

Determinants of Plasma Ascorbic Acid in a Healthy Male Population¹

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Abstract

The antioxidant properties of vitamin C may be involved in the prevention of cancer. The correlation between dietary vitamin C intake as estimated by a dietary questionnaire and plasma ascorbic acid (AA) was examined in 68 nonsmoking male volunteers aged 30-59 years. Determinants of plasma AA as well as interrelationships between various antioxidants in plasma were also explored. The determinants of plasma AA were examined by a multiple regression model containing dietary vitamin C, calories, body weight, and amount of beverages consumed. Higher vitamin C intake ($P < 0.0002$) increased plasma AA, while greater body weight ($P < 0.005$) decreased plasma AA. A significant correlation ($r = 0.43$; $P < 0.0003$) between vitamin C intake and plasma AA was observed. There was a negative correlation between plasma AA and plasma uric acid ($r = -0.32$; $P < 0.007$) and positive associations between plasma β -carotene and plasma α -tocopherol ($r = 0.39$; $P < 0.001$) and between plasma β -carotene and plasma glutathione peroxidase ($r = 0.32$; $P < 0.008$). Vitamin C supplement users had higher plasma AA compared to nonusers. The relationship between plasma AA and vitamin C intake appears to be curvilinear with the non-supplement users at the linear part of the curve and the supplement users at the plateau. Plasma AA is an appropriate biomarker, in our subjects, of dietary vitamin C except for people consuming large amounts of this vitamin either in their diet or in supplemental form.

Introduction

Cancer is the second leading cause of death in the United States. Doll and Peto (1) suggest that approximately 35% of cancer deaths could be attributed to dietary factors. Diets high in fruits and vegetables are associated with a decrease in the risk of cancers of the lung, breast, colon, rectum, esophagus, oral cavity, stomach, cervix, pancreas, larynx, and bladder (2-5). One of the major components of fruits and vegetables implicated in protection

against cancer is AA³ (6). The anticarcinogenic effect of AA may be due largely to its antioxidant properties (2).

Oxygen free radicals are thought to play an important role in developing or exacerbating diseases such as cancer (7-9). To counteract the action of free radicals, both in preventing formation of these oxidants as well as in repairing oxidative damage, groups of protective compounds exist in the body. In humans these protective compounds are components of the diet (e.g., vitamin C, tocopherol, and carotenoids), enzymes synthesized in the body (e.g., superoxide dismutase and glutathione peroxidase), and small molecules which are end products of metabolic pathways (e.g., bilirubin and urate; Ref. 10). The interrelationships among the various compounds are important in the overall ability of the organism to combat the damage caused by both endogenous oxidants produced by mitochondrial respiration and lipid peroxidation, and exogenous oxidants, which include natural dietary constituents, UV radiation, radon gas, and cigarette smoke.

Vitamin C is particularly effective as an antioxidant and free radical scavenger (7) because it is easily oxidized to DHAA, thus decreasing the oxidative or free radical damage to the cell and its constituents (10, 11). DHAA is enzymatically reduced back to ascorbate in RBC. The redox state of the plasma can also be influenced by transition metals (12) and antioxidant defenses, such as β -carotene (13), α -tocopherol (14, 15), glutathione peroxidase, and urate (16).

The level of plasma AA is influenced by life style as well as by antioxidants in the plasma. Some important factors affecting plasma AA are smoking (17-20), oral contraceptives (21), physical exercise (22-24), gender (25, 26), alcohol consumption (17, 27), age (24, 26, 28), and certain disease states (8, 29-33). An increase in plasma AA after supplementation with vitamin C has also been reported by numerous investigators (29, 34-36).

One of the major challenges for dietary assessment in nutritional epidemiology is accurate measurement of both long- and short-term intake of nutrients (37, 38). A reliable evaluation of nutritional status may be obtained by direct estimates of nutrient status through measurement of blood components, tissue, or body wastes. This information can complement as well as validate dietary questionnaires.

In the case of vitamin C, biological markers such as plasma AA or leukocyte AA may provide for reliable estimation that is not influenced by recall factors generally known to prejudice the dietary estimation of nu-

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³ The abbreviations used are: AA, ascorbic acid; DHAA, dehydroascorbate.

trients. Conversely, estimates of dietary vitamin C may serve as a surrogate for plasma AA in large-scale studies where the measurement of plasma AA is not feasible. Therefore, one of the aims of this study was to examine the relationship between dietary questionnaire estimates of vitamin C and plasma AA levels. As a methodological issue, we were interested in how the correlation between vitamin C intake and plasma AA varied depending on whether subjects with incomplete or erroneous questionnaire data were either removed from the analysis or reinterviewed for better data.

We were also interested in examining the relationship between AA and other antioxidants in the plasma because of the importance of these compounds in preventing free radical damage to the cell. Furthermore, we wished to determine how personal characteristics and life style factors influenced plasma AA.

Methods

Men aged 30–59 years were recruited from the greater Beltsville, Maryland area through advertisements in newsletters of local places of employment, newspapers, and fliers posted at local establishments. Screening procedures eliminated those who had smoked cigarettes within the past 6 months, who had chronic diseases, or who had dietary restrictions. For subjects eligible at this stage, blood and urine samples were collected, and medical evaluations were administered by a physician. All were within normal limits, and 68 subjects completed the entire study. Ethical approval for this study was obtained from the Human Volunteer Committees of the National Cancer Institute, United States Department of Agriculture, and Georgetown University.

At the beginning of the study the subjects completed an extensive questionnaire, the Health Habits and History Questionnaire (39). This provided estimates of usual dietary intake of energy and macro- and micronutrients, alcohol consumption, and vitamin supplement use in the 12 months preceding the study. The questionnaire also collected information about usual physical activity, smoking history, medical status, family history of cancer, occupational exposure to various materials, perceived stress, and social support systems.

Fasting blood samples were collected using EDTA as an anticoagulant. Samples were kept on ice until processing, a maximum of 30 min. Whole blood was centrifuged at $1800 \times g$ at 4°C for 10–15 min. For AA analysis the resulting plasma was stabilized with metaphosphoric acid and vortexed and stored at -70°C . For the other assays the plasma was stored at -70°C until analysis.

Total AA and DHAA were determined in plasma using a modification of the 2,4-dinitrophenylhydrazine method (40, 41). Duplicate portions of standard or sample were combined with 2,4-dinitrophenylhydrazine coupling reagent (with Cu^{2+} ions for total AA assay and without Cu^{2+} ions for DHAA assay). There is a high correlation between AA measured by high-performance liquid chromatography and 2,4-dinitrophenylhydrazine (42), and both of these methods provided results which were not significantly different when erythorbic acid was not present in the sample (43). Plasma α -tocopherol (44), retinol (44), and β -carotene were measured by high-performance liquid chromatography (45), glutathione peroxidase by the method of Paglia and Valentine (46), and uric acid by the modified Trinder method (47).

Table 1 Mean baseline characteristics of the study population

	Quartiles (by plasma AA)				Overall mean (SE)
	1st	2nd	3rd	4th	
<i>n</i>	17	17	17	17	68
Plasma AA ^a	0.63	0.94	1.15	1.39	1.03 (0.035)
Age (years)	42.4	40.7	40.4	39.0	40.6 (0.980)
Weight (kg)	87.2	76.3	81.5	78.5	80.9 (1.339)
Lean weight (kg)	68.3	62.4	65.1	65.7	65.6 (0.825)
Activity score ^b	8.1	8.2	12.2	11.8	10.0 (0.627)
Energy intake (kcal)	2209	2444	2401	2282	2334 (109.2)
Total vitamin C ^c	176	183	287	288	233 (28.0)
Diet C	121	150	178	169	155 (11.0)
Supplemental C	55	33	109	119	78 (23.1)
Plasma β -carotene ^d	19.6	22.3	19.8	30.7	23.1 (2.20)
Plasma uric ^e	6.4	5.8	6.0	5.6	6.0 (0.12)

^a AA in mg/dl.

^b Activity score ranged from 1 to 23. 1 = sedentary; 23 = athlete.

^c Vitamin C intake in mg/day.

^d β -Carotene in $\mu\text{g}/\text{dl}$.

^e Uric acid in mg/dl.

The data were analyzed using the SAS statistical software package (48). The interrelationship among the plasma antioxidants and the relationship between dietary vitamin C estimates and plasma AA were investigated using Pearson product-moment correlations. A regression model to analyze the determinants of plasma AA was developed. The variables examined were total dietary vitamin C, weight, lean body mass (by bioelectrical impedance), calories consumed, a physical activity score (the score ranged from 1 to 23: 1 = sedentary and 23 = athlete), beverage ingestion, a stress score (the score ranged from 1 to 5: 1 = felt stress every day and 5 = never or rarely felt stress), medical problems, percentage of calories from sweets and from alcohol, amount and type of fat, and race. Dietary vitamin C and calories were natural log-transformed to reduce skewness. Specific hypotheses which examined the difference between users and nonusers of vitamin C supplements were tested using regression models, correlations, and *t* tests.

Results

Characteristics of the study population are shown in Table 1, divided into quartiles ($n = 17$ in each quartile) by the plasma AA level at the time of entry into the study. The participants were males with an average age of 40.6 years (range, 30–59 years) and average weight of 80.9 kg (178 lbs). Subjects in the lowest plasma AA quartile tended to have higher lean weight and total weight and lower vitamin C intake than did subjects in the other quartiles. Total activity was also lower in this group of people. In contrast, plasma uric acid was highest in the subjects with the lowest plasma AA.

Various antioxidants in the plasma appear to be related to each other (Table 2). A negative correlation is observed between AA and uric acid ($r = -0.32$; $P < 0.007$). β -Carotene is positively correlated with α -tocoph-

Table 2 Correlations among plasma antioxidants

Antioxidant	Correlations ^a (Pearson)	P
Ascorbic acid		
Uric acid	-0.32	0.007
β -Carotene	0.23	0.055
β -Carotene		
α -Tocopherol	0.39	0.001
Glutathione peroxidase	0.32	0.008

^a $n = 68$ for all correlations.

erol ($r = 0.39$; $P < 0.001$) as well as with glutathione peroxidase ($r = 0.32$; $P < 0.008$) and marginally with AA ($r = 0.23$; $P < 0.055$).

All variables mentioned in the methods section were examined as potential determinants of plasma AA, but the final regression model consisted of total vitamin C intake, body weight, calories, and total amount of beverages consumed (Table 3). The calories variable was kept in the model to control for the amount of food ingested, and total amount of beverages consumed was a part of the model, since it appeared to affect plasma AA in vitamin C supplement users.

The main determinant of plasma AA was vitamin C ingested, which was significant at $P < 0.0002$. The same regression model was used to examine vitamin C supplement users and nonusers. The intake of vitamin C (as estimated by the questionnaire) is a strong determinant ($P < 0.0007$) of plasma AA among non-supplement users but is a weaker determinant among supplement users of

vitamin C ($P < 0.04$). The relationship between plasma AA and vitamin C intake for supplement users and nonusers is illustrated in Fig. 1. This indicates that within the group which consumes high amounts of vitamin C supplements, it is difficult to distinguish between those who have a moderately high intake as compared to those who have a still higher intake. Vitamin C intake produces a linear increase in plasma AA, which approaches a plateau above a certain intake.

The regression model shows a marginal negative association of AA with total body weight ($P < 0.062$) when all 68 subjects were analyzed together. However, one subject appeared to be different from the other subjects in that he was the second heaviest (97.8 kg) and had the highest plasma AA level on entry into the study, despite his not using vitamin C supplements and having lower-than-average dietary vitamin C intake. When this subject was excluded from the analysis the negative relationship became much stronger ($P < 0.005$). Furthermore, a significant ($P < 0.018$) relationship between plasma AA and body weight was also observed for non-supplement users. It may be that this subject had inaccurate questionnaire information or that the laboratory values were erroneous. It is also possible that this subject regulates AA differently from the norm, as has been reported in one study of guinea pigs (49).

The relationship between the vitamin C intake estimated by the diet questionnaire and plasma AA and DHAA is shown in Table 4. The total vitamin C intake (dietary vitamin C plus supplemental vitamin C) showed a reasonable correlation ($r = 0.43$; $P < 0.0003$) with plasma AA. Vitamin C supplement users and nonusers were then examined separately. There was a strong re-

Table 3 Determinants of plasma ascorbic acid using a regression analysis with vitamin C intake, body weight, calories, and beverages in the model

	Total vitamin C ^a intake (ln)	Weight ^b	Calories ^c (square root)	Beverages ^d	R-Square
All ($n = 68$)					
β^e	0.202	-0.006	-0.002	0.00003	0.24
P	0.0002	0.062	0.667	0.611	
Non-supp. user ^f ($n = 46$)					
β	0.301	-0.005	-0.002	0.00005	0.28
P	0.0007	0.178	0.698	0.532	
Supp. user ^f ($n = 22$)					
β	0.167	-0.009	-0.004	0.0002	0.36
P	0.040	0.124	0.678	0.028	
All ^g ($n = 67$)					
β	0.207	-0.008	0.0007	0.00001	0.32
P	0.0001	0.005	0.874	0.859	
Non-supp. user ($n = 45$)					
β	0.291	-0.008	0.0007	-0.00007	0.40
P	0.0001	0.018	0.880	0.243	
Supp. user ($n = 22$)					
β	0.167	-0.009	-0.004	0.0002	0.36
P	0.040	0.124	0.678	0.028	

^a Total vitamin C is dietary plus supplemental vitamin C.^b Body weight (kg).^c Daily energy intake estimated by questionnaire.^d Reported daily beverage intake (coffee, tea, soft drinks, alcohol, water, and soda, g/day).^e β are of natural log vitamin C intake and square root caloric intake.^f Non-supplement users are defined as taking less than 30 mg of supplement/day, and supplement users are defined as taking more than 30 mg of supplemental vitamin C/day.^g One subject removed from the analyses. See text for details.

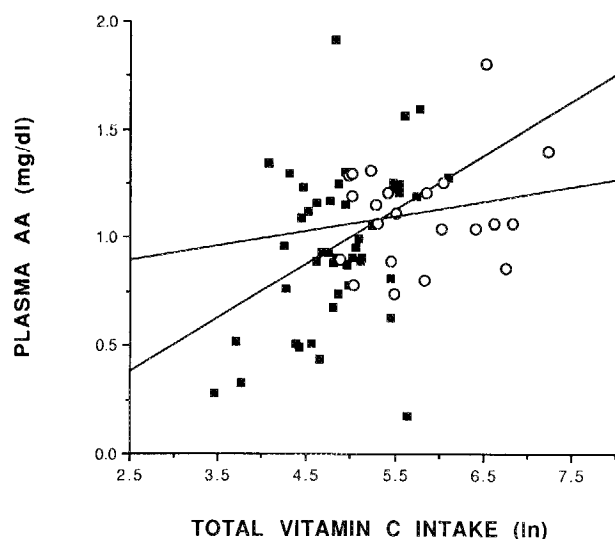


Fig. 1. Increase in plasma AA for total vitamin C intake in vitamin C supplement-users (O) and non-supplement users (■).

relationship between plasma AA and estimated dietary intake among non-supplement users. Among supplement users there was consistently higher plasma AA, but the correlation between intake and plasma within that subgroup was low, presumably due to a plateau effect.

A nonsignificant association between total vitamin C intake and DHAA ($r = 0.22$; $P < 0.077$) was observed. In contrast, a high correlation ($r = 0.70$; $P < 0.0001$) between plasma AA and DHAA was observed, implying that the concentration of DHAA in the plasma is dependent on the concentration of AA and only indirectly on the absolute amount of vitamin C in the diet.

To investigate whether improved information could be obtained from the questionnaire, subjects with incomplete or questionable answers were either removed from the analyses or reinterviewed (Table 5). Seven questionnaires were eliminated because the reported energy intake appeared spuriously high or low, and in a separate analysis six questionnaires were eliminated for various types of reporting errors (some examples are too few food items or over 95% of portion size reported as medium). Sixteen subjects were reinterviewed five months later and selectively queried about their previously questionable answers, to determine whether reinterviewing would correct major problems in their first questionnaires. There appears to be a slight improvement in the correlation between estimated dietary vitamin C intake and plasma AA when the six questionnaires with serious errors were removed. Eliminating outliers based on calorie consumption decreased the correlation between vitamin C intake and plasma AA. When subjects were reinterviewed instead of omitting questionnaires with errors, the correlation was lower for vitamin C intake and plasma AA.

Discussion

The results of this study show that certain antioxidants in the plasma are interrelated, that the major determinants of plasma AA are dietary vitamin C and body weight, and

Table 4 Correlations between diet questionnaire and plasma AA

	Correlations (Pearson)	P	Mean plasma AA mg/dl
Total vitamin C ^a * plasma AA			
All ($n = 68$)	0.43	0.0003	1.03
Non-sup. user ($n = 46$)	0.48	0.0006	0.98
Sup. user ($n = 22$)	0.20	0.376	1.12
Total vitamin C ^a * plasma DHAA			
All ($n = 68$)	0.22	0.077	
Plasma AA * plasma DHAA			
All ($n = 68$)	0.70	0.0001	

^a Total diet vitamin C includes dietary and supplemental vitamin C.

that questionnaire estimates of vitamin C correlated reasonably well with plasma AA.

Various antioxidants in the plasma appear to be related to each other, some positively and some negatively. A negative association is observed between plasma AA and uric acid. The mechanism controlling this relationship at the physiological level is at present unknown. However, this observation is similar to another finding in which serum uric acid decreased in elderly subjects after they were supplemented with high doses of vitamin C (50). There is some evidence that uric acid may conserve ascorbate in blood and other biological fluids (10). While ascorbate may be one of the more important antioxidants in plasma (7), a role of urate has also been suggested in free radical scavenging. Ames *et al.* (8) postulate that with the loss of the ability to synthesize ascorbate in higher primates, a concurrent loss occurred in uricase, an enzyme that breaks down urate. In higher primates urate is not broken down, and high concentrations are found in the plasma, as is the case in humans. With the presence of high amounts of urate in the plasma, this compound can play an important role in free radical scavenging. The relationship between uric acid and AA seen in this study is intriguing and needs to be followed up in more detail.

Other antioxidants in the plasma also appear to be positively related, notably β -carotene with α -tocopherol and glutathione peroxidase, and possibly β -carotene with AA. These relationships are interesting but need to be explored further, since they may have implications for interpreting studies investigating the role of antioxidants.

The two major determinants of plasma AA are total vitamin C intake (from dietary and supplemental sources) and body weight. As the intake of vitamin C increases, the plasma level of AA also rises. The rise in plasma level appears to be linear over the range of intakes seen among non-supplement users (approximately 30–210 mg/day). Supplement users tend to have higher plasma AA levels, but there is a weaker relationship between the vitamin C intake and plasma level of AA. Thus it appears that individuals taking supplemental vitamin C are reaching a level of plasma AA which produces a smaller increase in plasma AA for each increment in intake. This type of relationship has been described in other reports (51, 52). More information is needed on the short- and long-term pools of ascorbate in various tissues as the concentration of AA in cells and organs may increase even when the plasma level has reached a plateau.

Table 5 Correlation between total vitamin C and plasma AA for reinterviewed and with/without flagged subjects

	Questionnaire	Questionnaire ^a	Questionnaire ^b	Reinterviewed
Correlation	0.43	0.36	0.46	0.36
P<	0.0003	0.004	0.0002	0.002
n	68	61	62	68

^a Outliers on amount of calories consumed.

^b Error flag: people flagged for a number of errors and therefore eliminated.

High body weight is a negative determinant of plasma AA, implying that heavier people may need more vitamin C to attain the same plasma level as lighter people. This effect may be due to the dilution of AA, since the fluid volume is greater in larger persons, or to higher muscle mass in heavier persons, which acts as an AA "sink." As was seen with the relationship of vitamin C intake and plasma AA, the supplement users have a higher plasma AA and may have reached a level where a relationship between body weight and plasma level is not observed. Based on this finding, perhaps recommendations for vitamin C should be expressed according to body weight and not by absolute amounts. It has been suggested that separate Recommended Dietary Allowances for vitamin C should be considered for men and women (53). If the Recommended Dietary Allowances for vitamin C were based on weight, sex differences would also be taken into account.

The questionnaire estimate of dietary intake of vitamin C correlated reasonably well with plasma AA. However, the association between vitamin C intake and DHAA is weak, implying that plasma DHAA is not coming from the diet. This in itself may be interesting, since there is still a controversy about the existence of DHAA in plasma (54). DHAA may be an artifact of the assay, so that when higher levels of plasma AA are present, more DHAA is formed in the analysis process. The high correlation between plasma AA and DHAA in this study supports this hypothesis.

The questionnaire estimates of vitamin C appeared to be good, and reinterview of poor respondents did not improve the correlations between the dietary intake of vitamin C and plasma AA. This generalization can be made only for vitamin C estimates from this study.

In conclusion, there are relationships between various antioxidants in the plasma. A negative association was seen between AA and uric acid, and positive associations were found between β -carotene and α -tocopherol, β -carotene and glutathione peroxidase, and β -carotene and AA. In this study the major determinants of plasma AA are vitamin C intake and body weight, and plasma AA is an appropriate marker for questionnaire estimates of vitamin C intake.

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